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Recurrent linezolid-resistant *Enterococcus faecalis* infection in a patient with pneumonia

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SUMMARY

Objective: It has been reported that LZD-resistant Enterococcus in the gastrointestinal tract of mice colonizes persistently and shows variable minimum inhibitor concentration (MIC) values. However, the colonization characteristics of Enterococcus with LZD resistance in patients remain elusive. Here, we report the case of a patient with recurrent pneumonia due to infection with LZD-resistant *Enterococcus faecalis* strains. The colonization characteristics of the strains isolated from this patient were analyzed.
Methods: Ten *E. faecalis* strains were isolated from tracheal secretions obtained from the patient during five recurrences of pneumonia over the course of 10 months. Clonal relationships were determined by pulsed-field gel electrophoresis (PFGE) with *Sma*I-macrorestricted genomic DNA. The susceptibility of the isolates to LZD was determined by Etest in Mueller–Hinton agar.
Results: The homology of these strains was demonstrated by PFGE, suggesting that occult bacterial colonization by LZD-resistant *E. faecalis* is possible as late as a year after exposure to LZD. These strains showed variable MICs as determined by the Etest. LZD-resistant isolates contained single or double nucleotide mutations in domain V of 23S rRNA as confirmed by PCR and sequencing. The sensitivity of the strains to vancomycin was demonstrated by broth macrodilution, and vancomycin was an effective clinical treatment on each occasion.
Conclusions: Our results indicate that LZD-resistant *E. faecalis* strains may colonize persistently in vivo, leading to recurrent infection.

1. Introduction

Enterococcus faecalis is an opportunistic pathogen and a major cause of both community-acquired and nosocomial infections, including pelvic infections, endocarditis, neonatal infections, urinary tract infections, and respiratory infections.[1] The rise in prevalence of antibiotic-resistant enterococci, including vancomycin-resistant enterococci (VRE), has gained much attention in the clinical setting. Recently an increasing number of reports of linezolid (LZD)-resistant enterococci have emerged in the clinic.[2] Although resistance has been observed in enterococci without prior exposure to LZD, resistance is usually associated with prior and prolonged exposure.[3] LZD resistance mainly involves three mechanisms: mutations in the domain V region of the 23S rRNA gene, acquisition of the ribosomal methyltransferase gene *cfr*, and mutations in the *rplD* gene encoding the 50S ribosomal protein L4.[4,5] Genetic mutation in the central loop of the domain V region of the ribosome is frequently used to investigate LZD resistance in the clinical setting. *E. faecalis* contains four copies of rRNA operons (*rrn*) on its chromosome and the level of resistance to LZD is correlated with the number of copies of the 23S rRNA gene that are mutated.[5,6]

It has been reported that LZD-resistant Enterococcus in the gastrointestinal tract of mice colonizes persistently and shows variable minimum inhibitor concentration (MIC) values, as well as mutations in the domain V region of the 23S rRNA gene. However, the characteristics of the recurrent colonization by clinically isolated LZD-resistant Enterococcus remain elusive. In this study, sequential strains of *E. faecalis* isolated from a patient with recurrent respiratory infections were analyzed by pulsed-field gel

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electrophoresis (PFGE), and the dynamics of the MIC values and genetic mutations in the 23S rRNA V domain were investigated.

2. Materials and methods

2.1. Case report and bacterial isolates

A 52-year-old man diagnosed with acute leukemia in August 2007 and then receiving chemotherapy for many times. He was treated with a 5-day course of LZD at a dose of 600 mg twice daily in February 2010 for pneumonia caused by a suspected Gram-positive bacterium; this was his first treatment with LZD. His condition gradually improved, with a steady decrease in C-reactive protein (CRP) level and an improvement in physical appearance and radiological index. During this hospitalization, diverse antibiotic regimens were given before the use of LZD, including ceftriaxone, piperacillin–tazobactam, and meropenem. Cultures of blood and sputum specimens were consistently negative. The diagnosis of pneumonia was made on the basis of the case history, radiological findings, inflammation indicators, and the treatment outcome.

LZD treatment was administered on a second occasion, on January 11, 2011, again for the treatment of pneumonia. LZD-intermediate *E. faecalis* strains E1 and E2 were isolated from tracheal secretions on January 23 and 25, 2011, respectively. Vancomycin at a dose of 1000 mg twice daily was then used and continued for 5 days after his CRP level had decreased, culture of tracheal secretions had become negative, and his radiological index had improved. A smear test of the tracheal secretions revealed abundant neutrophils, five epithelial cells per low-power field, and sometimes Gram-positive cocci in chains on Gram stain. Culture of tracheal secretions further identified the presence of *E. faecalis*.

Subsequently, the patient suffered further episodes of pneumonia with confirmed *E. faecalis* infection of the tracheal secretions on another four occasions. During the five occurrences of pneumonia with pathogens identified, 10 *E. faecalis* strains were isolated from tracheal secretions. Details of the samples are listed in Table 1.

During the first four occurrences of pneumonia, intravenous vancomycin was continued for 5 days after the patient became afebrile, expectoration ended, and the sputum culture was recurrently negative; during the final episode on October 6, 2011, this antibiotic was continued for 7 days after the symptoms had alleviated and radiological findings had improved. Moreover, chest X-rays revealed an infiltrate with air bronchogram over the left middle field in the first three occurrences of pneumonia and over the right lower lung field in the last two occurrences. Recovery from pneumonia was confirmed by computed tomography for the last episode of Enterococcus-associated pneumonia. During this year of recurrent Enterococcus-associated pneumonia, the patient had stable-phase leukemia without neutropenia and did not receive chemotherapy. During a 2-year follow-up after the last occurrence, the patient did not suffer from pneumonia due to an *E. faecalis* infection.

Table 1
Genetic mutations and minimum inhibitory concentrations (MICs) of 10 *Enterococcus faecalis* strains in this study

Strain	MIC	Genetic mutations in domain V of 23SrRNA				
		1	2	3	4	Ratio
E1[a]	4	G2424U	W	W	W	1/4
E2[a]	4	G2424U	W	W	W	1/4
E3[b]	32	G2424U	W	W	G2576U	2/4
E4[b]	32	G2424U	W	W	G2576U	2/4
E5[c]	16	G2424U	W	W	G2576U	2/4
E6[c]	16	G2424U	W	W	G2576U	2/4
E7[d]	4	G2424U	W	W	W	1/4
E8[d]	4	G2424U	W	W	W	1/4
E9[e]	32	G2424U	W	W	G2576U	2/4
E10[e]	16	G2424U	W	W	G2576U	2/4

W, wild-type.
[a,b,c,d,e]The five instances of pneumonia: E1 and E2 were isolated on January 23 and 25, 2011, respectively; E3 and E4 were isolated on February 11 and 12, 2011, respectively; E5 and E6 were isolated on March 3 and 4, 2011, respectively; E7 and E8 were isolated on March 25 and 26, 2011, respectively; E9 and E10 were isolated on October 6 and 8, 2011, respectively.

2.2. Antibiotic susceptibility testing and PFGE

Susceptibility of the isolates to antibiotics commonly used against *E. faecalis* was measured by Etest (AB Biodisk, Solna, Sweden) with a 0.5 McFarland standard inoculum in Mueller–Hinton agar (bioMérieux, France), in accordance with the manufacturer's recommendations. Results suggested that the isolates were sensitive to ampicillin and vancomycin (by disk diffusion method). Clinical and Laboratory Standards Institute (CLSI) criteria and the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were applied to define the cut-off points for LZD. A MIC cut-off value of $\leq$2 μg/ml was assigned as sensitive, the median was 4 μg/ml, and resistance was defined as $\geq$8 μg/ml. The MICs of vancomycin were determined by broth macrodilution as per CLSI guidelines. For quality control purposes, the vancomycin-sensitive *E. faecalis* reference strain ATCC 29212 was used. Clonal relationships were determined by pulsed-field gel electrophoresis (PFGE) with *Sma*I-macrorestricted genomic DNA, as reported previously.[5]

2.3. PCR amplification of individual 23S rRNA genes

The domain V region (bp 2254–2683) of four different copies (rr1, rr2, rr3, and rr4) of the 23S rRNA gene was amplified separately and sequenced from the *E. faecalis* isolates in accordance with a previously reported protocol.[5] Briefly, genomic DNA from *E. faecalis* isolates was extracted using the Lysis Buffer for Microorganism to Direct PCR (TAKARA, Japan). Four different copies (rr1, rr2, rr3, and rr4) of domain V of the 23S rRNA gene were amplified separately from *E. faecalis* isolates using primers reported previously.[5] Subsequently, the amplified PCR products of the four different copies (rr1, rr2, rr3, and rr4) were separated by agarose (1%) gel electrophoresis and the individual bands were extracted and gel purified (Qiagen, Germany). The domain V region (bp 2254–2683) of each 23S rRNA gene was sequenced with the primer 5′- GGCGCTGGTGGGATACTACCC-3′.[5]

3. Results

PFGE typing results demonstrated a high homology of the 10 *E. faecalis* isolates (Figure 1), suggesting occult colonization by LZD-resistant or intermediate *E. faecalis* in vivo. During the course of this study, the patient was hospitalized in different wards under strict surveillance for hospital-acquired infection and so we concluded that the *E. faecalis* infections of this patient were not hospital-acquired. Table 1 summarizes the variable MIC values and genetic mutations in domain V of 23S rRNA of these isolates. Certain isolates, including E1, E2, E7, and E8, showed intermediate resistance to LZD and contained a single G2424U mutation. The resistant isolates, including E3–E6, E9, and E10, contained double mutations at G2424U and G2576U in this region, indicating that LZD-resistant strains can emerge in the host even 10 months after final exposure to LZD. All strains showed sensitivity to vancomycin by broth macrodilution, and the patient recovered after each treatment with vancomycin.

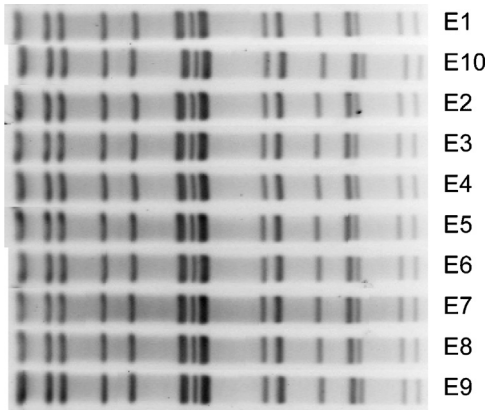

Figure 1. Pulsed-field gel electrophoresis banding pattern of 10 *Enterococcus faecalis* isolates.

4. Discussion

LZD-resistant *E. faecalis* with genetic mutations in domain V of 23S rRNA often emerge in the host after exposure to LZD. Here, the recurrent infection with LZD-resistant *E. faecalis* may have been due to the repeated administration of LZD during the early phase of pneumonia. LZD resistance is often explained by genetic mutations in domain V of 23S rRNA when selective pressure is applied, however it remains unclear how long the resistant strain can colonize in vivo. In this study, the recurrent detection in tracheal secretions of LZD-intermediate or resistant *E. faecalis* occurred for a year after final exposure to LZD and detection of the resistant strain. Moreover, all isolates in this study showed high homology, as demonstrated by PFGE, suggesting long-term colonization and difficulty in eradicating LZD-resistant strains in vivo even after 1 year.

There are two possible explanations for the difficulty in eliminating the LZD-resistant isolates in this patient. The patient suffered from acute leukemia which would affect his ability to recover from pneumonia. Under these conditions, even a course of vancomycin or other antibiotics is insufficient to eliminate the LZD-resistant strains. Additionally, LZD-resistant *E. faecalis* is more difficult to eradicate and patients are more susceptible to recurrent flare-ups as compared to patients infected with LZD-sensitive bacteria.[7,8] Under normal conditions, the MIC of LZD-resistant *E. faecalis* gradually decreases over time after exposure to LZD has ended. In the current study, however, isolates with fluctuating MIC values were detected in the patient. We speculate that *E. faecalis* colonized the host and different strains with high homology resulted in different MIC values and genetic mutations in domain V of the 23S rRNA gene due to LZD exposure. These colonies reside in the respiratory tract, oropharyngeal isthmus, or other parts of the host. These strains recurrently flare up to a pathogenic state at different times under specific conditions. We found that *E. faecalis* infection in this patient could be controlled by vancomycin, and with prolonged vancomycin treatment the infection was cleared, suggesting that prolonged treatment may facilitate the elimination of LZD-resistant Enterococcus.

In conclusion, our study describes the characteristics of colonizing LZD-resistant *E. faecalis* in vivo and demonstrates the importance of monitoring recurrent flare-ups from infection with this type of strain. Our findings may also help define the best therapeutic strategies to eradicate LZD-resistant *E. faecalis* in the clinical setting.

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Ethical approval: Signed informed consent was obtained from the patient for publication of the clinical data.

Conflict of interest: No conflict of interest to declare.

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